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Ab initio Simulations of Cu Binding Sites in the N-Terminal Region of PrP

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The prion protein (PrP) binds Cu^{2+} ions in the octarepeat domain of the N-terminal tail up to full occupancy at pH=7.4. Recent experiments show that the HGGG octarepeat subdomain is responsible for holding the metal bound in a square planar coordination. By using first principle *ab initio* molecular dynamics simulations of the Car-Parrinello type, the Cu coordination mode to the binding sites of the PrP octarepeat region is investigated. Simulations are carried out for a number of structured binding sites. Results for the complexes Cu(HGGGW)+(wat), Cu(HGGG) and the dimer $[Cu(HGGG)]_2$ are presented. While the presence of a Trp residue and a H_2O molecule does not seem to affect the nature of the Cu coordination, high stability of the bond between Cu and the amide Nitrogens of deprotonated Gly's is confirmed in the case of the Cu(HGGG) system. For the more interesting $[Cu(HGGG)]_2$ dimer a dynamically entangled arrangement of the two monomers, with intertwined N-Cu bonds, emerges. This observation is consistent with the highly packed structure seen in experiments at full Cu occupancy.

1 Introduction

The Prion Protein (PrP) is a cell surface glycolipid protein, highly expressed in the central nervous system of many mammals. Its physiological rôle is still unclear, but it has been shown that it can selectively bind Cu^{2+}. A number of Cu binding sites (no less than four) have been identified along the whole protein.

Mature PrP (comprising a.a. 23-231) has a flexible, disordered, N-terminal (23-120) and a globular C-terminal (121-231). The N-terminal domain of human PrP contains four repeats of the eight peptides, PHGGGWGQ, from residue 60 to 91, called *octarepeat* [a]. It has been shown that each octarepeat is able to bind one Cu^{2+} ion [b].

Cu-octarepeat interaction is cooperative, and could possibly have a rôle in disease related PrP aggregation. Several techniques have been used in the study of the octarepeat structural arrangements, including X-ray crystallography[1] and EXAFS spectroscopy[2]. Experiments have shown that only the oligopeptide HGGGW is directly involved in Cu^{2+} coordination. The crystallographic data shows that the Cu^{2+} ion is penta-coordinated, displaying a square planar equatorial coordination with three Nitrogens and one Oxygen from the HGGGW peptide, and an axial Oxygen from a water molecule.

The equatorial coordination of the N3O1 type is with the $\delta 1$ Nitrogen of the His, the deprotonated amide Nitrogens of the two following Gly's and one carbonyl Oxygen from

[a] In other species this number may be different. For instance, *Bos gaurus*, has 5 octarepeats.
[b] Other possible binding sites have been found in the C-terminal region.

the second Gly of the sequence. The axially bound water molecule is kept in position by a hydrogen bond to the H $\epsilon 1$ of the Trp indole ring.

We have investigated the coordination mode of Cu to the octarepeat via *ab initio* molecular dynamics simulations *à la* Car–Parrinello. In order to have a system of tractable size, we have not considered the whole octarepeat, but only smaller portions of it, complexed with Cu and water in various combinations. More precisely we have investigated the following systems: the Cu(HGGGW)(H_2O) complex, a box containing Cu(HGGG) and 41 water molecules and a system composed by two HGGG oligopeptides, both in the presence and in the absence of an associated pair of Cu^{2+} ions.

The downsizing of the system here consistent with the experimental findings that only the HGGGW portion is directly involved in Cu^{2+} coordination[1,3].

2 Methods

Car–Parrinello MD simulations have been carried out employing the Quantum-ESPRESSO package[4]. We have used Vanderbilt's ultrasoft pseudopotentials and the PBE exchange-correlation functional[5]. Periodic boundary conditions have been imposed on the super-cell, with a minimum separation of 5 and 8 Å between replica, for neutral and charged systems, respectively. The energy cutoff was of 25 Ry, while the hard cutoff for the augmented charge density was of 250 Ry. All the simulations were spin-restricted. In some particularly interesting cases, namely the monomer Cu(HGGG) and the dimer $[Cu(HGGG)]_2$, we have performed spin unrestricted simulations, with S=1/2 and S=1, respectively.

Every simulation consisted of the following steps.

1. Electronic energy minimization with fixed atomic positions.
2. Energy minimization with respect to atomic and electronic degrees of freedom to attain the equilibrium geometry.
3. Two subsequent molecular dynamics simulations of 0.25 ps each at $T = 100$ and 200 K, respectively [c].
4. Molecular dynamics simulations of about 2 ps at $T = 300$ K, using the same thermostat as in 3.

Thermalization is necessary to slowly approach room temperature and avoid that temperature oscillations obscure the electronic properties of the ground state. Equations of motion have been integrated using the usual velocity-Verlet algorithm with a time step of 0.12 fs. Simulations have been carried out on Linux-clusters using 8-16 processors, depending on the size of the systems [d]. The CPU time per step was, on average, of 9.6 s when running on 16 nodes at 2.7 GHz.

3 Results

Simulations of Cu(HGGGW)(H_2O) show that the water molecule in the axial coordination site is not bonded to Cu, but rather it is hydrogen-bonded to the Trp side chain. Indeed we

[c] A Nosé-Hoover thermostat at the required temperature was coupled to ionic degrees of freedom.
[d] For a reference, the monomer Cu(HGGG) comprises 49 atoms and 157 valence electrons.

don't see a propensity for a chemical Cu-O(water) bond. Crystallographic packing effects can explain the presence of water seen in the crystal. We have performed simulations in super-cells of increasing size, finding that the volatility of the water molecule actually increases. We have thus decided to discard the Trp from further simulations.

Analysis of several trajectories of the complex Cu(HGGG) at $T = 300$ K show that the N3O1 coordination is stable, with the bond between Cu and amide Nitrogens from deprotonated Gly's stronger than that between Cu and the $\delta 1$ Nitrogen of the His. The Cu-O bond with the carbonyl group of the second Gly is weak, and affected by the greater mobility of the peptide C-terminal.

Simulations of Cu(HGGG) immersed in a box with 41 water molecules confirm the picture in which deprotonation of Gly's is favored by the greater stability of the bond with Cu, even if at physiological pH Gly's are expected to be protonated.

Study of the system $[Cu(HGGG)]_2$ in vacuum shows that, on the time scale of the ps, formation of a dimer is possible when the tetrapeptides bind copper. Exchange of ligands (visible after 0.86 ps at $T = 300$ K) between the two Cu ions is seen to keep the two tetra-peptides close to each other. Also for the dimer we observe a preference for an N amidic binding, while the bond with the imidazole ring of the His can be broken. In spin restricted simulations we observe a rather small Cu-Cu average distance (from 2.1 to 3 Å) which, in spin unrestricted ones, becomes slightly larger (2.4 to 3.4Å), and thus nearer to experimental observations. In the absence of Cu, long-range electrostatic and dispersive interactions are unable to keep the two (HGGG) close together. We have not yet studied the effects of hydrophobic interactions.

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